local structure of an element in the native tertiary structure formation, we examined the structural alternation of staphylococcal nuclease (SNase) upon the direct structural modulation of the element 3. To introduce the azobenzene linkage into SNase, we prepared a double cysteine mutant, E57C/K64C. The mutated sites are in the $\alpha 1$ helix of SNase and in the element 3 (Lys62-Glu67). The crosslinking reaction was carried out and the products were examined by TOF-MS. The products include two crosslinked species, in which the molar ratios of SNase and azobenzene are 1:1 and 1:2. After the ion-exchange chromatography, we succeeded in isolating the 1:1 specie. Upon UV LED (367 nm) light irradiation, the UV-Vis spectral change of the azobenzene linked SNase was observed, which is similar to that of azobenzene alone. While the cis form of the azobenzene linked SNase showed the native like CD spectrum, after blue light irradiation to form the trans form, significant CD spectral change was clearly observed. The results indicate that photoisomerization of azobenzene can directly modulate the local structure of the structural element 3 and the modulation influences the native tertiary structure.

3P-048 誘導折り畳み反応の中値解析: スタフィロコッカスクレアーゼ変異体の誘導折り畳み反応におけるリガンド結合の役割

Φ -value analysis of induced folding: the role of the ligand binding in induced folding of disordered staphylococcal nuclease mutant

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There are many reports about disordered proteins which can fold with the aid of binding to their interaction partners, called induced folding. The studies of the induced folding give a new insight into both protein folding and molecular recognition. We previously reported that the $\Delta 140$ -149 staphylococcal nuclease obeys the binding before folding scheme; $D+L \leftrightarrow DL \leftrightarrow FL$ (D:disordered state, F:folded state, L:ligand). To characterize the structure of DL, we prepared Ala mutants of $\Delta 140$ -149, and performed the Φ -value analysis by kinetic CD measurements. The $\Delta \Delta G_{DL}$ and $\Delta \Delta G_{TS}$ upon the Ala mutation were estimated by the K_d of the DL obtained from the burst phase amplitude and the k_f from the relaxation rate analysis, respectively. The mutants involved in the binding site (T82A, K84A, Y85A, Y113A, V114A, and Y115A) showed relatively high Φ -values (0.4 to 0.8) and the Φ -values of the other positions are close to 0. In the transition state between DL and FL, additional structural formation was expected by the increases of Φ -values. These results suggest that the binding site with the ligand serves as a folding nucleus. We reported that the W140 lacking mutant loses the non-local interaction between the N- and C- terminal sub-domains. The ligand in the DL can compensate the lost interaction to recover the foldability.

3P-049 電子常磁性共鳴分光法による天然変性 Staphylococcal Nuclease 変異体の構造解析

Structural Characterization of the Intrinsically Disordered Mutants of Staphylococcal Nuclease by Electron Paramagnetic Resonance Spectroscopy

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Two Staphylococcal nuclease mutants, $\Delta 140$ -149 and 33A34 are unstructured under physiological conditions, but can fold into native structures with the aid of ligand binding. While 33A34 displays folding before binding, $\Delta 140$ -149 exhibits binding before folding. The CD spectra of these mutants under physiological conditions are slightly different from each other. It can be assumed that the selectivity of the two schemes of induced folding partly depends on the denatured structures. To characterize the denatured structures, we applied electron paramagnetic resonance (EPR) spectroscopy to these mutants. In order to generate site-directed labeling, we prepared K24C and K133C in both the $\Delta 140$ -149 and 33A34. In the $\Delta 140$ -149, the spectrum due to the position 24 displays broad splitting near the hyperfine extremum, resembling the WT. However, the splitting disappears in the 33A34, indicating a loss of structure in the vicinity of position 24. These results suggest that $\Delta 140$ -149 partly forms the native-like β -core structure, but 33A34 does not. On the other hand, although a somewhat broad hyperfine structure can be seen at position 133 of the WT, this position displays sharper peaks at the hyperfine extremum in both the $\Delta 140$ -149 and 33A34, indicating that the C-terminal regions of both $\Delta 140$ -149 and 33A34 are more disordered relative to the WT. Consequently, the residual structure within the N-terminal β -barrel is presumed to play an early role in the ligand binding in the disordered state of $\Delta 140$ -149.

3P-050 網羅的アラニン挿入変異解析法を用いたジヒドロ葉酸還元酵素の機能発現及び構造形成領域の抽出

Extraction of the regions encoded foldability and/or functionality

from Dihydrofolate reductase by the systematic alanine insertion

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To design an artificial protein, it is essential to elucidate the building blocks of a foldable and functional polypeptide. We applied the systematic alanine insertion analysis to Dihydrofolate reductase (DHFR) to extract the building blocks, called functional and structural elements, required for the functional expression and the structural formation. So far the functional and structural elements of DHFR were qualitatively assigned by the TMP resistance assay and the solubility assay. In order to examine the properties of the functional and structural elements of DHFR, we examined the structures and the enzymatic activities of the alanine insertion mutants quantitatively. The alanine insertion mutants into one of the structural element (72nd-86th residues) of DHFR were purified. We revealed that the effect of insertion can be classified into two types: One loses the structure (Type I), and the other loses the stability (Type II). To evaluate the structure of the Type II mutants, we examined the fluorescence of 1-anilino-naphthalene-8-sulfonic acid (ANS). The type II mutants exhibit the native-like CD spectra. The ANS fluorescent spectra indicated that the Type II mutants clearly show the substantial increase of the fluorescence intensity, indicating that the Type II mutants are in equilibrium between the native and disordered structures. The results of activity assay will be also presented in the meeting.

3P-051 網羅的アラニン挿入変異解析による Human p100 SNase 様ドメインの構造形成に関わる領域の抽出

Extraction of the structural element of SNase-like domain in Human p100 by Alanine Insertion Analysis

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Staphylococcal nuclease (SNase) is a model protein for the studies of protein folding and stability. We demonstrated that the elements responsible for the function and the foldability, called functional and structural elements, respectively, can be extracted by the systematic alanine insertion analysis of SNase.

Recently, a transcriptional regulator protein, p100, was found in human. The crystal structure of the C-terminal domain (residues 654-870, called TSN domain) showed that an additional domain called Tudor domain is inserted between the $\beta 2$ and $\beta 7$ of the SNase-like (SN) domain. Although the sequence identity between SNase and the SN domain of TSN is only 15%, their tertiary structures are almost identical.

To identify the structural elements of the SN domain and compared them with those of SNase, we prepared alanine insertion mutants of the construct comprised of the SN-domain alone (SND), in which an alanine is inserted into every interval on the sequence of the $\alpha 2$ helix. The $\alpha 2$ helix corresponds to the $\alpha 1$ helix of SNase involving a structural element. CD spectra indicated that the alanine insertion into the C-terminal part of the helix leads to the loss of the structure, while the mutants of the other part exhibit a native-like CD spectrum. The region is almost overlapped with the structural element of SNase. From these observations, it can be supposed that the structural elements are preserved in both SND and SNase.

3P-052 HMGB2 タンパク質の構造安定性に関する解析

Analysis of structure stability of HMGB2

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High mobility group B2 (HMGB2) protein is a eukaryotic chromosomal one that binds into the DNA minor groove without significant sequence specificity, producing a strong bend in the DNA backbone. HMGB2 is believed to facilitate transcription and has been shown to promote and stabilize DNA loop complexes. To analyze relationship between structure and function of HMGB2 protein, we investigated the secondary structure and physicochemical property of HMGB2 protein by using circular dichroism spectroscopy (CD). Firstly, cDNA of HMGB2 was cloned into pET28a vector and transformed into *Escherichia coli* BL21(DE3) cells. Then, the protein was purified from this protein expression system by using affinity chromatography and anion exchange chromatography. Under various environmental conditions, the CD spectra of HMGB2 were measured. The little changes in CD spectra of HMGB2 were shown with pH change from 4 to 10, suggesting the stability of structure of HMGB2 against pH change of solution. Next, we investigated the changes in the CD spectra over a temperature range of 5-100 °C to analyze thermal stability of the structure. Remarkable change in HMGB2 spectrum pattern occurred at 50°C. As increasing the temperature over 50°C, the content of random coil dramatically increased. This structural change according the increase of temperature was reversible. We will analyze the secondary structure of HMGB2 in the presence of DNA and discuss the relation between structure and function of HMGB2.